

The status of Iranian oomycete species in GenBank

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Zusammenfassung: Oomyceten sind sehr vielfältige pilzähnliche Mikroorganismen (*Chromista*), die Menschen, Pflanzen, Insekten, Algen, Tiere, Pilze und sogar andere Oomyceten befallen können. Nahezu 900 Arten dieser umfangreichen Gruppe wurden weltweit identifiziert und anschließend wurden mehrere Arten aus dem Iran berichtet. Die meisten Arten wurden nur anhand der Morphologie identifiziert und ihre DNA-Informationen (Sequenzen) wurden nicht an GenBank übermittelt. Im Hinblick auf neue Empfehlungen in der Pflanzen- und Pilztaxonomie sollten alle untersuchten Isolate phylogenetisch analysiert und die Sequenzen in GenBank hinterlegt werden, auch, weil die Zugangsnummern in den entsprechenden Manuskripten benötigt werden. Bis jetzt sind fünf Gattungen iranischer Oomyceten, darunter *Pythium* (30 Arten), *Phytophthora* (5 Arten), *Phytophthora* (24 Arten), *Saprolegnia* (10 Arten) und *Achlya* (5 Arten) in GenBank verfügbar. Hier werden eine kurze Checkliste der gültigen Oomyceten-Arten und Details zum Status der Arten in GenBank diskutiert.

Abstract: *Oomycetes* are highly diverse fungal-like microorganisms (*Chromista*) that could infect humans, plants, insects, algae, animals, fungi and even other oomycetes. Near to 900 species of this extensive group were identified in the world and subsequently several species were reported from Iran. Most of the species were identified based on morphology only and their DNA information (sequences) was not submitted to GenBank. Regarding new recommendations in plant and fungal taxonomy, all studied isolates should be analyzed phylogenetically and sequences deposited in GenBank, implying that retrieved accession numbers are necessary in relevant manuscripts. Until now, five genera of Iranian Oomycetes including *Pythium* (30 species), *Phytophthora* (5 species), *Phytophthora* (24 species), *Saprolegnia* (10 species) and *Achlya* (5 species) are available in GenBank. Here, a brief checklist of valid oomycete species and details of the status of the species in GenBank will be discussed.

Oomycetes (Syn. *Oomycota* and *Peronosporomycetes*) are fungal-like microorganisms belonging to the eukaryotic phylum *Chromista* (CAVALIER-SMITH 1981, DICK 2001). Traditional taxonomy in oomycetes was generally based on morphological characteristics and morphometric data, and classified them as filamentous, coenocytic, sporangia and zoospore-forming fungal-like microorganisms (*Phycomycetes*). Similarity between oomycetes and true fungi arise from convergent evolution and presence of the same hyphal structures, life styles, e.g., saprotrophic or parasitic on different substrates in soil and water environments (TOJO & al. 2012). Some morphological structures such as sporangia, oogonia and antheridia are highly variable and absent in many species

(LEVESQUE & DE COCK 2004). With the advent of new molecular genetic techniques, several changes occurred in the systematics of oomycetes (SPARROW 1960). Sequences of ITS-rDNA have been widely applied when studying oomycetes, but the region does not have enough variation in even morphologically separate species (DE COCK & al. 2008, KAGEYAMA & al. 2007). Therefore, scientists suggest using several loci (e.g., *coxI*, *coxII*, LSU-rDNA, SSU-rDNA) for oomycetes as concatenated phylogenetic tree based on a consolidated species concept (BIARD & al. 1995, UZUHASHI & al. 2009, CHENARI BOUKET & al. 2015). According to the International Code of Nomenclature (TURLAND & al. 2018), since 1-Jan-2013 mycologists have to register new taxa with the relevant data in one of the three main online deposits e.g., Index Fungorum, MycoBank of Fungal Names. It is recommended to add the accession numbers of sequences which have been submitted to GenBank or another international sequence database (Unite) to forthcoming papers (HAWKSWORTH 2011). Here, we want to briefly present the status of Iranian oomycetes in GenBank.

List of oomycetes

A list of the oomycetes with sequences that have been submitted to GenBank was compiled. Most of the quoted sequences are related to studies of Iranian phytopathologists, although a part of them was deposited by foreign scientists. For each genus, several identities such as number of isolates, submitted loci, published/direct submission and substrates have been organized in three heat-maps.

Results

One and/or several loci of 76 species of the five genera *Pythium*, *Phytophythium*, *Phytophthora*, *Saprolegnia* and *Achlya* are presently uploaded in GenBank (4. November 2020). *Pythium* is present with 32 species, namely *P. abapressorium*, *P. aphanidermatum*, *P. attrantheridium*, *P. carolinianum*, *P. catenulatum*, *P. debaryanum*, *P. dissotocum*, *P. ershadii*, *P. glomeratum*, *P. hydnosporum*, *P. inflatum*, *P. iranense*, *P. kando-vanense*, *P. kashmirensense*, *P. longipapillum*, *P. monoclinum*, *P. nunn*, *P. oligandrum*, *P. oopapillum*, *P. oryzicollum*, *P. paroecandrum*, *P. porphyrae*, *P. plurisporium*, *P. pyrillo-bum*, *P. pyrriosporum*, *P. rhizo-oryzae*, *P. spinosum*, *P. ultimum*, *P. urmianum*, *P. sylvaticum*, *P. viniferum* and *Pythium* sp. (ABRINBANA & al. 2016; BADALI & al. 2016, 2020; BADRBANI & al. 2018; CHENARI BOUKET & al. 2015, 2016a, b, c; GHALAMFARSA & BOLBOLI 2014; SALMANINEZHAD & GHALAMFARSA 2019). ITS-rDNA, *coxI*, *coxII* and β -*tubulin* are the amplified loci (Fig. 1A). Soil, rice, cereals, cucumber, watermelon, squash, ryegrass, hordeum and vegetation rhizosphere are the substrates from which *Pythium* species were recovered (Fig. 1B). Status of published/direct submission is always presented in part C of the respected figures.

Five species of *Phytophythium*, namely *Pp. litorale*, *Pp. oedochilum*, *Pp. ostracodes*, *Pp. vexans* and *Phytophythium* sp. are present in GenBank (CHENARI BOUKET & al. 2016d, ROBIDEAU & al. 2011). ITS-rDNA and *coxI* are the amplified loci (Fig. 2A). Rice, ivy, almond and *Juncus* sp. are the substrates from which *Phytophythium* species were recovered (Fig. 2B).

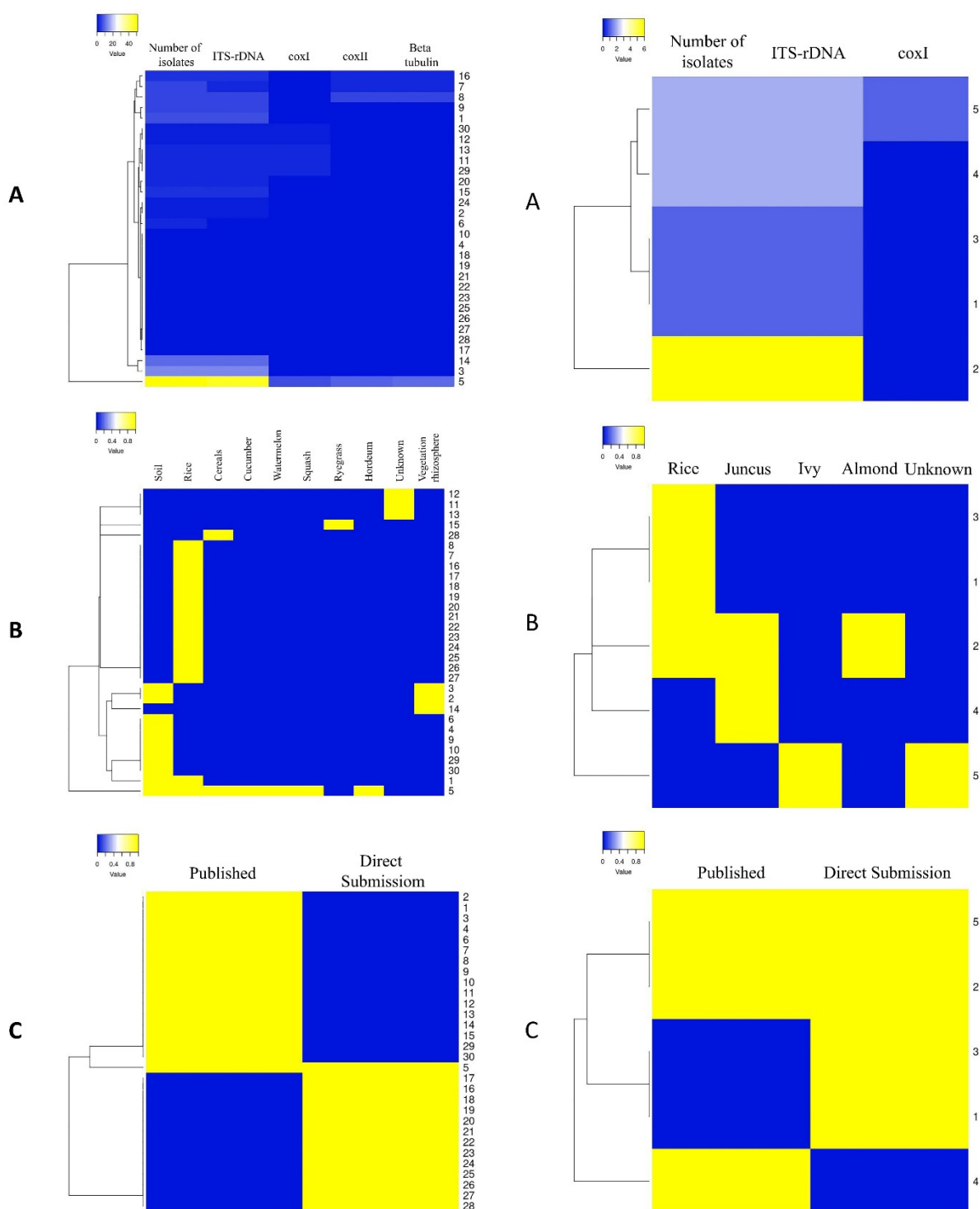


Fig. 1. Left. Status of *Pythium* in GenBank. A Number of isolates and amplified loci, B Substrates, C status of publication. (1 *P. nunn*, 2 *P. glomeratum*, 3 *P. viniferum*, 4 *P. attrantheridium*, 5 *Pythium* sp., 6 *P. carolinianum*, 7 *P. oryzicollum*, 8 *P. longipapillum*, 9 *P. ultimum*, 10 *P. paroecandrum*, 11 *P. ershadii*, 12 *P. pyriosporum*, 13 *P. urmianum*, 14 *P. sylvaticum*, 15 *P. kandovanense*, 16 *P. plurisporum*, 17 *P. hydno sporum*, 18 *P. debaryanum*, 19 *P. inflatum*, 20 *P. rhizo-oryzae*, 21 *P. pyrilobum*, 22 *P. kashmirens*, 23 *P. catenulatum*, 24 *P. oopapillum*, 25 *P. dissotocum*, 26 *P. porphyrae*, 27 *P. aphaniderm*, 28 *P. oligandrum*, 29 *P. monoclinum*, 30 *P. iranense*)

Fig. 2. Right. Status of *Phytophythium* in GenBank. A Number of isolates and amplified loci, B) Substrates, C) status of publication. (1 *Phytophythium* sp., 2 *Pp. littorale*, 3 *Pp. ostracodes*, 4 *Pp. oedochilum*, 5 *Pp. vexans*).

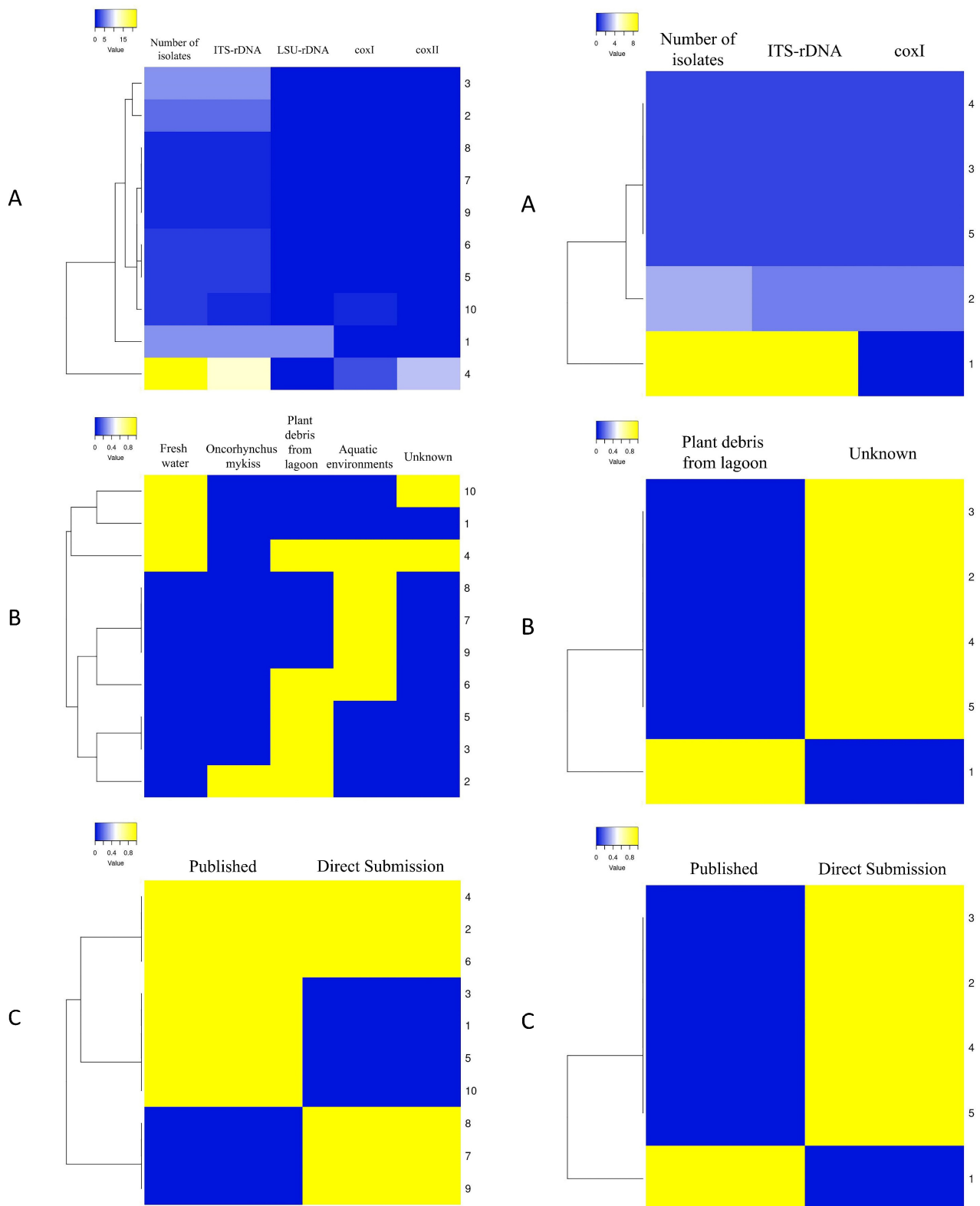


Fig. 3. Left. Status of *Saprolegnia* in GenBank. *A* Number of isolates and amplified loci, *B* Substrates, *C* status of publication. (1 *S. maragheica*, 2 *S. parasitica*, 3 *S. diclina*, 4 *S. ferax*, 5 *S. anisospore*, 6 *Saprolegnia* sp., 7 *S. anomalies*, 8 *S. australis*, 9 *S. mixta*, 10 *S. bulbosa*)

Fig. 4. Right. Status of *Achlya* in GenBank. *A* Number of isolates and amplified loci, *B* Substrates, *C* status of publication. (1 *Achlya* sp., 2 *A. bisexualis*, 3 *A. recurva*, 4 *A. conspicua*, 5 *A. flagellata*)

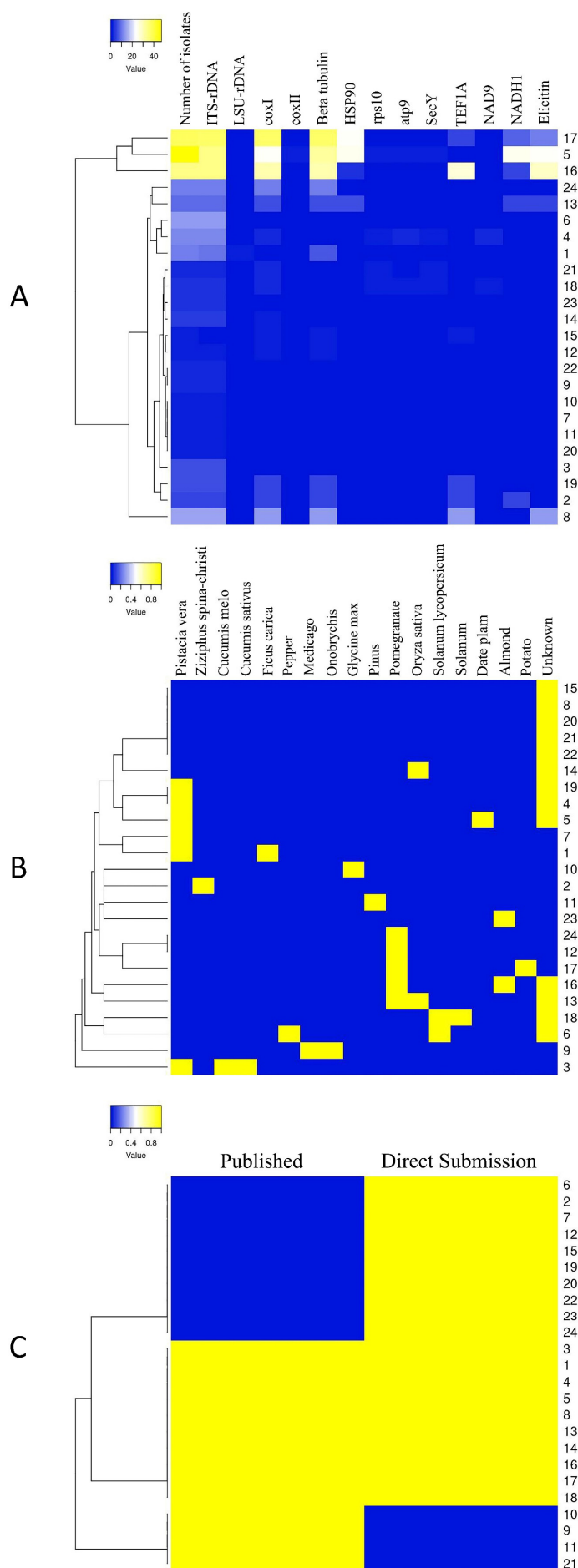


Fig. 5. Status of *Phytophthora* in GenBank. *A* Number of isolates and amplified loci, *B* Substrates, *C* status of publication. (1 *P. parsiana*, 2 *Ph. taxon banhashemiana*, 3 *Ph. melonis*, 4 *Ph. pistaciae*, 5 *Phytophthora* sp., 6 *Ph. capsica*, 7 *Ph. taxon walnut*, 8 *Ph. drechsleri*, 9 *Ph. medicaginis*, 10 *Ph. sojae*, 11 *Ph. megasperma*, 12 *Ph. kelmanii*, 13 *Ph. pseudocryptogea*, 14 *Ph. inundata*, 15 *Ph. lateralis*, 16 *Ph. cryptogea*, 17 *Ph. erythroseptica*, 18 *Ph. iranica*, 19 *Ph. palmivora*, 20 *Ph. austrocedrae*, 21 *Ph. parsiniana*, 22 *Ph. tropicalis*, 23 *Ph. cactorum*, 24 *Ph. cinnamomi*).

Saprolegnia has ten species, i.e. *S. anisospora*, *S. anomalis*, *S. australis*, *S. bulbosa*, *S. diclina*, *S. ferax*, *S. maragheica*, *S. mixta*, *S. parasitica*, *Saprolegnia* sp.) in GenBank (LIU & al. 2018, MASIGOL & al. 2020). ITS-rDNA, LSU, *coxI* and *coxII* are the amplified loci for the *Saprolegnia* spp. (Fig. 3A). Fresh water, aquatic environments, plant debris from lagoon and *Onchorhynchus mykiss* are the substrates from which *Saprolegnia* spp. were recovered (Fig. 3B).

The genus *Achlya* has five species, namely *A. bisexualis*, *A. conspicua*, *A. flagellate*, *A. recurva*, and *Achlya* sp. present in GenBank (MASIGOL & al. 2020). ITS-rDNA, LSU, *coxI* and *coxII* are the amplified loci for *Saprolegnia* spp. (Fig. 4A). Fresh water, aquatic environments, plant debris from lagoon and *Onchorhynchus mykiss* are the substrates from which *Saprolegnia* species were recovered (Fig. 4B).

Phytophthora has 24 species, namely *Ph. austrocedrae*, *Ph. cactorum*, *Ph. capsici*, *Ph. cinnamomi*, *Ph. cryptogea*, *Ph. drechsleri*, *Ph. erythroseptica*, *Ph. inundata*, *Ph. iranica*, *Ph. kelmania*, *Ph. lateralis*, *Ph. medicaginis*, *Ph. megasperma*, *Ph. melonis*, *Ph. palmivora*, *Ph. parsiana*, *Ph. parsiniana*, *Ph. pistaciae*, *Ph. pseudocryptogea*, *Ph. sojae*, *Ph. tropicalis*, *Ph. taxon banihashemiana*, *Ph. taxon walnut* and *Phytophthora* sp. present in GenBank (MIRABOLFATHY & al. 2001; SAFAIEFARAHANI & al. 2015, 2016; GHALAMFARSA & al. 2008, 2010; ROBIDEAU & al. 2011). ITS-rDNA, LSU, *coxI*, *coxII*, β -tubulin, *HSP90*, *rps10*, *atp9*, *SecY*, *TEF1A*, *NAD9*, *NADH1*, Elicitin and *rps10* are the amplified loci for *Phytophthora* spp. (Fig. 5A). Almond, *Cucumis melo*, *C. sativus*, date palm, *Ficus carica*, *Glycine max*, *Medicago*, *Onobrychis*, *Oryza sativa*, pepper, *Pistacia vera*, *Pinus*, pomegranate, potato, *Solanum lycopersicum*, *Solanum* and *Ziziphus spina-christi* are the substrates that *Phytophthora* species are recovered from them (Fig. 5B).

Discussion

Iran is one of the countries in the world with most extensive ecological niches and with diverse climate regions. Therefore, a rich biodiversity is expected. But few studies were conducted about this diversity (KIANI & al. 2017) and especially about oomycetes, their distribution and host ranges or substrates in Iran. Based on the submitted sequences of Iranian oomycetes there are five genera with 74 species reported from Iran until now. Among them some species, such as *Pythium kandovanense*, *P. iranense*, *P. monoclinum*, and *Saprolegnia maragheica* were new species when submitted (BADALI & al. 2020, CHENARI BOUKET & al. 2015, LIU & al. 2018). Some others were new records for the fungi of Iran (e.g. *Pythium sylvaticum*, *P. abapressorium*) (CHENARI BOUKET & al. 2016b, c). Previously, there were several papers published in local journals solely based on morphological features and the availability of type specimens or ex-type cultures is not clear (CHENARI BOUKET & al. 2013). Regarding extensive overlapping of morphology among oomycetes, correct identification of such isolates without DNA sequencing is dubious. Absolutely, it does not mean that old studies on oomycetes are not acceptable. They are valuable and we may rely on them but should validate with polyphasic taxonomy including sequencing. Molecular genetic studies with ITS-rDNA are common

in oomycetes but this locus is not variable enough to distinguish the species (UETA & TOJO 2016). Therefore, using more loci, at least two, even better more, and performing a multi-locus strategy to produce concatenated phylogenetic trees is a strong and successful way (CHENARI BOUKET & al. 2015). In most cases, in the Iranian oomycete isolates just the barcoding locus ITS-rDNA was amplified. It shows that there is a gap to meet consolidated species concept standards. Scientists have to be encouraged to go ahead in phylogenetic analysis of several loci in their studies. When using GenBank, another challenge are the missing updates of the many sequences merely included as “sp.”, i.e. unidentified, despite later available publications giving the correct taxa for the isolates. Scientists should notify GenBank, when papers with the correct names are published to that the sequence entries can be updated.

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